Salicylate Downregulates 11-HSD1 Expression in Adipose Tissue in Obese Mice and in Humans, Mediating Insulin Sensitization

Citation for published version:
Nixon, M, Wake, DJ, Livingstone, DE, Stimson, RH, Esteves, CL, Seckl, JR, Chapman, KE, Andrew, R & Walker, BR 2012, 'Salicylate Downregulates 11-HSD1 Expression in Adipose Tissue in Obese Mice and in Humans, Mediating Insulin Sensitization' Diabetes , vol. 61, no. 4, pp. 790-796. DOI: 10.2337/db11-0931

Digital Object Identifier (DOI):
10.2337/db11-0931

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published In:
Diabetes

Publisher Rights Statement:
Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. See http://creativecommons.org/licenses/by-nc-nd/3.0/ for details.

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
S
alcylate anti-inflammatory agents, including ace
tylsaclic acid (aspirin) and salicylsalicylic acid
(salsalate) (1), improve insulin sensitivity in ani
mal models (2-5) and in healthy or obese humans
(6-9) and improve glycemic control in patients with type 2
diabetes (10-12). The mechanism of insulin sensitiza
tion is uncertain and may involve blockade of inhibitor of
κB kinase-β and, hence, nuclear factor-κB effects (13,3,14)
and/or interference with phosphorylation and activity of
CCAT enhancer binding protein-β (a transcription factor
involved in metabolic and inflammatory pathways) (15,16),
with associated reductions in proinflammatory cytokines.

11β-Hydroxysteroid dehydrogenase type 1 (11B-HSD1) catalyzes the regeneration of active cortisol from inert
cortisone (corticosterone from 11-dehydrocorticosterone
catalyzes the regeneration of active cortisol from inert
11β-HSD1–deficient mice and C57Bl/6 mice with sodium salicylate
for 4 weeks. Glucose tolerance was assessed in vivo. Tissue tran
script levels were assessed by quantitative PCR and enzyme activity
by incubation with 3H-steroid. Two weeks’ administration of salsalate was also investigated in a randomized double-blind pla
cebo-controlled crossover study in 16 men, with measurement of liver
11β-HSD1 activity in vivo and adipose tissue 11β-HSD1 tran
script levels ex vivo. In C57Bl/6 DIO mice, salicylate improved
glucose tolerance and downregulated 11β-HSD1 mRNA and activity
selectively in visceral adipose. DIO 11β-HSD1–deficient mice were resistant to these metabolic effects of salicylate. In men,
salicylate reduced 11β-HSD1 expression in subcutaneous adipose, and in vitro salicylate treatment reduced adipocyte 11β-HSD1 ex
pression and induced adiponectin expression only in the presence of 11β-HSD1 substrate. Reduced intra-adipose glucocorticoid re
generation by 11β-HSD1 is a novel mechanism that contributes to the metabolic efficacy of salicylates. Diabetes 61:790-796, 2012

Recent trials show salicylates improve glycemic control in type 2
diabetes, but the mechanism is poorly understood. Expression of the
glucocorticoid-generating enzyme 11B-hydroxysteroid dehy
drogenase type 1 (11B-HSD1) in adipose tissue is increased in vitro by proinflammatory cytokines and upregulated in obesity.
11β-HSD1 inhibition enhances insulin sensitivity. We hypothesized that salicylates downregulate 11β-HSD1 expression, contributing to their metabolic efficacy. We treated diet-induced obese (DIO) 11β-HSD1–deficient mice and C57Bl/6 mice with sodium salicylate
for 4 weeks. Glucose tolerance was assessed in vivo. Tissue tran
script levels were assessed by quantitative PCR and enzyme activity
by incubation with 3H-steroid. Two weeks’ administration of salsalate was also investigated in a randomized double-blind pla
cebo-controlled crossover study in 16 men, with measurement of liver
11β-HSD1 activity in vivo and adipose tissue 11β-HSD1 tran
script levels ex vivo. In C57Bl/6 DIO mice, salicylate improved
glucose tolerance and downregulated 11β-HSD1 mRNA and activity
selectively in visceral adipose. DIO 11β-HSD1–deficient mice were resistant to these metabolic effects of salicylate. In men,
salicylate reduced 11β-HSD1 expression in subcutaneous adipose, and in vitro salicylate treatment reduced adipocyte 11β-HSD1 ex
pression and induced adiponectin expression only in the presence of 11β-HSD1 substrate. Reduced intra-adipose glucocorticoid re
generation by 11β-HSD1 is a novel mechanism that contributes to the metabolic efficacy of salicylates. Diabetes 61:790-796, 2012

Salicylate Downregulates 11β-HSD1 Expression in Adipose Tissue in Obese Mice and in Humans,
Mediating Insulin Sensitization

Mark Nixon, Deborah J. Wake, Dawn E. Livingstone, Roland H. Stimson, Cristina L. Esteves,
Jonathan R. Seckl, Karen E. Chapman, Ruth Andrew, and Brian R. Walker

RESEARCH DESIGN AND METHODS

Chemicals were purchased from Sigma-Aldrich (Dorset, U.K.) unless otherwise
stated.

Cell culture experiments. Simpson-Golabi-Behelien syndrome (SGBS) cells are a human preadipocyte cell line characterized by a high capacity for in vitro
differentiation (30). Cells were cultured in Dulbecco’s modified Eagle’s medium
(F12; supplemented with 10% (v/v) FCS, 50 units/mL penicillin per 50 units/mL
streptomycin, biotin (33 μM/L), and pantothentic acid (17 μM/L) at 37°C in
5% CO2. Adipogenic differentiation was induced after reaching near conflu
ence as described previously (30). At day 14, cells were incubated for 24 h
in stripped serum medium prior to treatment with salicylic acid (10, 30, and
100 μM/L) or vehicle (0.1% ethanol) for 24 h, and RNA was extracted for
analysis of 11β-HSD1 transcript. To assess the interaction between salicylate
and 11β-HSD1 in determining adiponectin (AdiQ) transcript levels, day 14 cells
were incubated for 24 h in cortisol-free, stripped serum medium prior to
treatment with cortisol (0.1 μM/L), cortisone (0.1 μM/L), or vehicle (0.1% ethanol)
with or without salicylic acid alone (100 μM/L), or vehicle (0.1% ethanol)
(0.1% ethanol) for 24 h. RNA was extracted for analysis.

Experiments in mice. Experiments were performed under license from the U.K.
Home Office. Mice were maintained under controlled conditions of light (6700–
1900 h) and temperature (18–20°C) and allowed access to food and drinking
water ad libitum. Adult male C57Bl/6 mice were obtained (Harlan Olac,
Oxfordshire, U.K.) at age 12 weeks. C57Bl/6 Lean animals were maintained on
normal chow diet (2.27% fat, 4.06% sucrose; 501151; Special Diet Services). Diet
induced obese C57Bl/6 mice (C57Bl/6 DIO) were given 10 weeks of high-fat diet
(58% fat, 12% sucrose; D12331; Research Diets, New Brunswick, NJ) before

From the Endocrinology Unit, Centre for Cardiovascular Science, University of
Edinburgh, Queen’s Medical Research Institute, Edinburgh, Scotland, U.K.
Corresponding author: Mark Nixon, m.nixon@ed.ac.uk.
Received 4 July 2011 and accepted 21 December 2011.
DOI: 10.2337/db11-0931
© 2012 by the American Diabetes Association. Readers may use this article as
long as the work is properly cited, the use is educational and not for profit,
and the work is not altered. See http://creativecommons.org/licenses/by
-nc-nd/3.0/ for details.
For human adipose tissue, 100 mg tissue was homogenized and RNA extracted using the QIAGEN RNaseA Lipid Tissue Mini kit (West Sussex, UK.) and quantified using the Ribogreen quantitation kit (Molecular Probes, Eugene, OR). RNA integrity was verified on a 1.2% agarose/Tris borate/EDTA gel. Oligo dT-primed cDNA was synthesized from 0.5 μg RNA using Promega Reverse Transcription System (Promega, Southampton, U.K.), together with controls from which reverse transcriptase was omitted. Transcript quantification was performed as above for mouse samples. Primer-probe sets for cyclophilin A and 11β-HSD1 were purchased from Applied Biosystems (Cheshire, U.K.). Random primers were used for 18S. The results are expressed as a ratio to the mean of cyclophilin A and 18S, as internal controls that did not differ between groups.

**RESULTS**

**Salicylate improves glucose tolerance in obese but not lean mice.** Ten weeks of high-fat feeding caused diet-induced obesity in male C57Bl/6 DIO mice, with elevated fasting plasma insulin levels compared with C57Bl/6 Lean mice (Table 1). On glucose tolerance testing, C57Bl/6 DIO mice were hyperglycemic and hyperinsulinemic compared with C57Bl/6 Lean mice, indicating development of insulin resistance (Table 1 and Fig. 1).

Four weeks of treatment with salicylate (120 mg/kg/day) had little measurable effect in C57Bl/6 Lean mice, except for elevated fasting plasma insulin. In C57Bl/6 DIO mice, salicylate decreased both fasting and postprandial plasma glucose levels (Table 1 and Fig. 1). Furthermore, there was a trend to reduced plasma triglyceride levels after salicylate treatment in C57Bl/6 DIO mice (P = 0.059) (Table 1). Salicylate downregulates visceral adipose 11β-HSD1 in DIO mice. Salicylate significantly reduced 11β-HSD1 mRNA in omental adipose tissue in C57Bl/6 DIO mice, with a similar trend in mesenteric adipose (P = 0.057) (Fig. 2A). In mesenteric adipose of C57Bl/6 DIO mice, salicylate also reduced 11β-HSD1 enzyme activity (Fig. 2B).

**11β-HSD1 deficiency blocks insulin-sensitizing effects of salicylate.** HSD1KO-DIO mice were protected against fasting hyperinsulinemia compared with weight-matched C57Bl/6 DIO controls. However, HSD1KO-DIO mice were still hyperinsulinemic, both fasting and postprandial, compared with C57Bl/6 Lean mice (Table 1 and Fig. 1). In marked contrast to C57Bl/6 DIO mice, salicylate had no effects on biochemical indices in HSD1KO-DIO mice (Table 1 and Fig. 1).

**11β-HSD1 deficiency prevents salicylate-induced changes in adipose transcript levels.** In C57Bl/6 DIO mice (Fig. 3), salicylate altered mRNA levels of several genes. In mesenteric adipose (Fig. 3B), salicylate increased AdiQ and decreased TNF-α, monocyte chemoattractant protein-1 (MCP-1), adipose triglyceride lipase (ATGL), and angiotensinogen (AGT) mRNA levels. This...
pattern of salicylate-induced changes was similar in omental adipose (Fig. 3A). With the exception of increasing AdiQ mRNA levels, these effects were absent in subcutaneous adipose tissue (Fig. 3C). Compared with salicylate-treated C57Bl/6 DIO mice, HSD1KO-DIO mice had a similar pattern of transcript changes in visceral adipose, with increased AdiQ and reduced TNF-α and ATGL mRNA levels (Fig. 3). However, in HSD1KO-DIO mice, salicylate treatment did not alter mRNA levels of any of the genes affected in C57Bl/6 DIO mice (Fig. 3).

**Salsalate downregulates adipose 11β-HSD1 in vivo in men.** Characteristics of the 16 male participants are shown in Table 2. Salsalate levels in plasma averaged 134 ± 33 mg/L during active treatment and were undetectable during placebo. Only 1 of the participants reported tinnitus during salsalate therapy, and there were no changes in indices of insulin sensitivity or lipid profile. Nevertheless, salsalate reduced 11β-HSD1 mRNA levels in subcutaneous adipose, an effect which was unrelated to BMI (Fig. 4) or plasma salsalate levels (data not shown). This was accompanied by reduced transcript levels for MCP-1 (33.6 ± 5.6% suppression, \( P < 0.02 \)), but not TNF-α or AdiQ. The effect of salsalate on 11β-HSD1 activity appeared restricted to adipose tissue since there was no change in first pass conversion of cortisone to cortisol in liver or in the ratio of cortisol to cortisone metabolites in urine (Fig. 4).

**Salicylate-induced upregulation of AdiQ is mediated by reduced 11β-HSD1 in human adipocytes.** In fully differentiated human SGBS adipocytes, 24-h incubation with salicylic acid in the absence of steroid dose-dependently reduced 11β-HSD1 mRNA levels (Fig. 5A) but had no effect on AdiQ mRNA (Fig. 5B). Both cortisol and cortisone treatment (0.1 μmol/L for 24 h) reduced mRNA

### Table 1

Effects of salicylate on body weight and fasting plasma biochemistry in mice, with biochemical indices, age, high-fat diet duration, and weight measurements for cohorts of mice

<table>
<thead>
<tr>
<th></th>
<th>C57Bl/6 Lean</th>
<th></th>
<th>C57Bl/6 DIO</th>
<th></th>
<th>HSD1KO-DIO</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>Salicylate</td>
<td>Vehicle</td>
<td>Salicylate</td>
<td>Vehicle</td>
<td>Salicylate</td>
</tr>
<tr>
<td>Weight at start (g)</td>
<td>28.8 ± 0.6</td>
<td>29.2 ± 0.5</td>
<td>38.4 ± 1.1</td>
<td>39.7 ± 1.7</td>
<td>38.0 ± 0.8</td>
<td>38.3 ± 0.5</td>
</tr>
<tr>
<td>Weight at end (g)</td>
<td>29.2 ± 0.8</td>
<td>29.5 ± 0.7</td>
<td>39.0 ± 1.4</td>
<td>40.3 ± 2.3</td>
<td>38.0 ± 0.8</td>
<td>39.0 ± 0.8</td>
</tr>
<tr>
<td>Age at end (weeks)</td>
<td>17.0 ± 0.0</td>
<td>17.0 ± 0.0</td>
<td>24.0 ± 0.0</td>
<td>24.0 ± 0.0</td>
<td>22.7 ± 3.3</td>
<td>22.7 ± 3.3</td>
</tr>
<tr>
<td>Time on HFD (weeks)</td>
<td>0</td>
<td>0</td>
<td>10.0 ± 0</td>
<td>10.0 ± 0</td>
<td>10.8 ± 0.8</td>
<td>10.8 ± 0.8</td>
</tr>
<tr>
<td>Fasting plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin (ng/mL)</td>
<td>0.95 ± 0.11</td>
<td>1.45 ± 0.20*</td>
<td>2.69 ± 0.28###</td>
<td>2.74 ± 0.32</td>
<td>2.01 ± 0.15##</td>
<td>2.65 ± 0.45</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>112.5 ± 7.8</td>
<td>113.2 ± 7.1</td>
<td>131.7 ± 12.2</td>
<td>109.0 ± 5.7*</td>
<td>119.5 ± 4.4</td>
<td>119.3 ± 7.5</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.59 ± 0.04</td>
<td>0.63 ± 0.04</td>
<td>0.83 ± 0.04##</td>
<td>0.70 ± 0.05</td>
<td>0.84 ± 0.05</td>
<td>0.81 ± 0.04</td>
</tr>
</tbody>
</table>

Data are mean ± SEM for \( n = 7–8 \) per group. Comparisons were by two-way ANOVA with Bonferroni post hoc tests. HFD, high-fat diet. *\( P < 0.05 \) vs. vehicle of same group. ###\( P < 0.01 \) vs. C57Bl/6 Lean vehicle. ####\( P < 0.001 \) vs. C57Bl/6 Lean vehicle. \( \phi P < 0.05 \) vs. C57Bl/6 DIO vehicle.

**FIG. 1.** Effects of salicylate on insulin sensitivity in C57Bl/6 Lean and DIO mice. Plasma glucose (A and C) and insulin (B and D) after intraperitoneal glucose injection in C57Bl/6 Lean, C57Bl/6 DIO, and HSD1KO-DIO mice. Area under the curve (AUC) for all groups shown for glucose (C) and insulin (D). Data are mean ± SEM for \( n = 7–8 \) per group for individual time points. Comparisons for AUC were by two-way ANOVA with Bonferroni post hoc tests. **\( P < 0.01 \) vs. vehicle in same group; #\( P < 0.05 \), ##\( P < 0.01 \) vs. Lean vehicle; \( \phi P < 0.05 \) vs. C57Bl/6 DIO vehicle.**
levels of AdiQ. In the presence of cortisone, coinubcation with salicylic acid attenuated the suppression of AdiQ, whereas salicylate had no effect on suppression of AdiQ by cortisol (Fig. 5B).

**DISCUSSION**

These results identify a novel mechanism by which salicylates may enhance insulin action in diet-induced obesity, involving downregulation of adipose 11β-HSD1 expression. This downregulation occurs selectively in adipose tissue depots in mice and in humans in vivo, is likely mediated directly within adipose, and is mimicked in human adipocytes in culture. It is crucial that mice deficient in 11β-HSD1 display similar changes in vivo metabolism and intra-adipose gene expression as salicylate-treated wild-type mice, and salicylate has no additional effects on these parameters in the absence of 11β-HSD1, implicating 11β-HSD1 as a mediator of the beneficial effects of salicylate.

The magnitude and components of the metabolic effects of salicylate remain somewhat contentious. For example, in humans, in addition to reports of improved insulin sensitivity (10,6,41,7), several reports describe worsening of insulin resistance (42–44) or attribute improved metabolism to an increase in insulin concentrations (9). Here, we confirm variable efficacy of salicylate in different settings. In lean mice, salicylate modestly increased plasma insulin concentration but had no other metabolic effects. In DIO mice, salicylate lowered fasting plasma glucose concentrations and improved glucose tolerance, as well as reducing 11β-HSD1 in visceral adipose (omental and mesenteric) but not subcutaneous adipose tissue. These effects, together with the lack of change in plasma insulin concentrations, are consistent with insulin sensitization primarily in visceral adipose tissue due to reduced local glucocorticoid regeneration by 11β-HSD1 (45,46), with secondary improvements in insulin sensitivity in other organs potentially mediated by altered adipokine secretion. The HSD1KO-DIO mice were <2 weeks younger than the C57Bl/6 DIO mice, so it is highly unlikely that age-dependent effects are responsible for the discrepancies in responses to salicylate between groups. In contrast, in the human study, although we did not undertake sensitive measurements of insulin sensitivity, such as euglycemic clamps, there were no measured effects of salsalate on fasting insulin or lipid profile; this was the case even if we examined only obese participants (not shown). It may be that high doses are required to induce robust insulin sensitization in humans: the salicylate concentrations achieved in this study (mean 134 mg/L, ≈1 mmol/L) (Table 2) are lower than in some other studies (e.g., 270 mg/L) (41). This inconsistency is in keeping with a mechanism of action mediated by 11β-HSD1 downregulation, since recent phase 2 clinical trials of selective 11β-HSD1 inhibitors demonstrate that metabolic effects are of relatively small magnitude and not always statistically significant (26,47). Previous studies demonstrating efficacy of salicylates in rodents use genetic models of obesity, including Zucker obese rats and ob/ob mice (32,2,18,3). To our knowledge, this is the first study to demonstrate the insulin-sensitizing effects of salicylates in vivo.

---

**FIG. 2. Effects of salicylate on 11β-HSD1 in DIO mice.** 11β-HSD1 mRNA expression in tissues from C57Bl/6 DIO mice (A). 11β-HSD1 enzyme activity in mesenteric and subcutaneous adipose tissue from C57Bl/6 DIO mice (B). Om, omental; Mes, mesenteric; SC, subcutaneous. Data are mean ± SEM for n = 4–8 per group. Comparisons were by Student unpaired t tests. *P < 0.05, **P < 0.01 vs. vehicle in same tissue.

**FIG. 3. Effects of salicylate on adipose mRNA levels in mice.** mRNA levels were measured for AdiQ, TNF-α, MCP-1, lipoprotein lipase (LPL), adipose triglyceride lipase (ATGL), and angiotensinogen (AGT) by qPCR in omental (A), mesenteric (B), and subcutaneous (C) adipose tissue from C57Bl/6 DIO and HSD1KO-DIO mice. Data are mean ± SEM for n = 4–8 per group. Comparisons were by two-way ANOVA with Bonferroni post hoc tests. *P < 0.05, **P < 0.01 vs. vehicle of same genotype; ♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂♂료
expression also observed in vivo. The downregulation of 11β-HSD1 by salicylates mediates the altered AdiQ expression also observed in vivo. The downregulation of 11β-HSD1 was selective for the visceral adipose depots in mice and, thus, ensured these HSD1KO-DIO mice were markedly hyperinsulinemic compared with lean mice. The striking lack of efficacy of salicylate treatment in HSD1KO mice is therefore consistent with downregulation of 11β-HSD1 playing a key role in its mechanism of action.

Downregulation of 11β-HSD1 by salicylates is likely to reflect a direct mechanism in adipose tissue, given its replication in an adipocyte cell line. Furthermore, we have demonstrated in vitro that salicylic acid reverses cortisone-mediated suppression of the insulin-sensitizing adipokine AdiQ, since salicylic acid had no effect when the contribution of 11β-HSD1 was negated by removal of steroid or by addition of cortisol, these data indicate that inhibition of 11β-HSD1 by salicylates mediates the altered AdiQ expression also observed in vivo. The downregulation of 11β-HSD1 was selective for the visceral adipose depots in mice but was evident in subcutaneous adipose in humans. Central adipose depots exhibit greater variation in lipolytic activity and more intense proinflammatory responses during high-fat feeding and are more glucocorticoid responsive (49). However, in humans, recent evidence suggests that 11β-HSD1 regenerates more cortisol in subcutaneous than in visceral adipose tissue (50), suggesting species-specific differences. Moreover, we did not find evidence that changes in proinflammatory cytokines, such as TNF-α, mediate the effects of salicylates on 11β-HSD1 expression; the downregulation of TNF-α by salicylates in C57Bl/6 DIO mice, but resistance to this effect in 11β-HSD1KO mice, suggests that effects of salicylates on intra-adipose inflammation may be a consequence rather than cause of downregulation of 11β-HSD1. This is further supported by the downregulation of TNF-α in the visceral adipose of 11β-HSD1KO mice compared with C57Bl/6 mice. In human adipose, TNF-α mRNA

| TABLE 2 Effects of salsalate on anthropometry and biochemistry in participating men |
|----------------------------------|----------------|----------------|
| Placebo                         | Salsalate      |
| Age (years)                     | 40.1 ± 10.0    | 37.6 ± 10.8    |
| BMI (kg/m²)                     | 31.9 ± 8.0     | 32.0 ± 8.0     |
| Waist-to-hip ratio              | 0.93 ± 0.23    | 0.94 ± 0.23    |
| Percent body fat                | 25.9 ± 6.5     | 26.1 ± 6.5     |
| Systolic BP (mmHg)              | 136.8 ± 34.2   | 137.9 ± 34.5   |
| Diastolic BP (mmHg)             | 79.7 ± 19.9    | 81.9 ± 20.5    |
| Fasting plasma                  |                |
| Salicylate (mg/L)               | ND             | 134 ± 33*     |
| Triglycerides (mmol/L)          | 1.5 ± 0.4      | 1.3 ± 0.3      |
| Total cholesterol (mmol/L)      | 4.7 ± 1.2      | 4.4 ± 1.1      |
| LDL cholesterol (mmol/L)        | 2.7 ± 0.7      | 2.6 ± 0.6      |
| HDL cholesterol (mmol/L)        | 1.3 ± 0.3      | 1.3 ± 0.3      |
| Glucose (mmol/L)                | 5.2 ± 1.3      | 5.1 ± 1.3      |
| Insulin (mU/L)                  | 26.5 ± 6.6     | 23.1 ± 5.8     |

Data are mean ± SEM for n = 16 per group. Comparisons were by Student paired t tests. BP, blood pressure; ND, not detected. *P < 0.01 vs. placebo.

FIG. 4. Effect of salsalate on 11β-HSD1 in humans. Subcutaneous adipose tissue 11β-HSD1 mRNA levels (A); the correlation of change in adipose mRNA levels after salsalate with BMI (B); liver 11β-HSD1, measured as appearance of cortisol in plasma after overnight dexamethasone suppression and administration of 25 mg cortisone by mouth at time 0 (C); and urinary cortisol metabolites (D) in 16 participants in a double-blind randomized crossover trial comparing salsalate with placebo. Data are mean ± SEM. Comparisons were by paired Student t test (A and D), Pearson correlation (B), or two-way repeated-measures ANOVA (C). *P < 0.02 vs. placebo. AU, arbitrary unit.
did not change in association with altered 11β-HSD1 mRNA. In the absence of cytokine-mediated regulation, more detailed investigation will be required to dissect the molecular mechanisms behind the salicylate-mediated regulation of 11β-HSD1 expression.

In conclusion, these findings suggest that the anti-inflammatory agent salsalate alters glucocorticoid metabolism in mice and humans in a pattern that differs between liver and subcutaneous adipose tissue. Downregulation of intra-adipose 11β-HSD1 may contribute to the insulin-sensitizing effect of salicylates.

ACKNOWLEDGMENTS

This work was supported by grants from the British Heart Foundation, the Wellcome Trust, and the Medical Research Council.

Within the past 2 years, J.R.S. and B.R.W. have consulted for AstraZeneca, Incyte, Roche, and Wyeth; received lecture fees from Merck; and received research funding from AstraZeneca and Wyeth. J.R.S. and B.R.W. are inventors on relevant patents held by the University of Edinburgh. No other potential conflicts of interest relevant to this article were reported.

M.N. conducted the experiments, analyzed data in mice and in vitro, and wrote the manuscript. D.E.L. and C.L.E. conducted the experiments and analyzed data in mice and in vitro. D.J.W., R.H.S., and R.A. conducted the experiments and analyzed data in humans. J.R.S. and K.E.C. contributed to discussion. B.R.W. wrote the manuscript, which was reviewed by all authors. B.R.W. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Parts of this study were presented at ENDO 2010 93rd Annual Meeting and Expo of The Endocrine Society, San Diego, California, 19–22 June 2010, and at the British Endocrine Society Meeting, Manchester, U.K., 15–18 March 2010.

The authors thank the Wellcome Trust Clinical Research Facility staff for facilitating the study; Jill Harrison, Alison McNeilly, Carolynn Cairns, Lynne Ramage, and Ash Taylor (all of University of Edinburgh) and the staff of the Clinical Biochemistry Laboratory, Western General Hospital, for technical assistance; and the staff of the Biomedical Research Facility, University of Edinburgh, for help with animal care. SGBS cells were kindly provided by Prof. Martin Wabitsch, University of Ulm, Germany, and Dr. Kerry McInnes, University of Edinburgh.

REFERENCES

3. Yuan M, Konstantopoulos N, Lee J, et al. Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikk-

beta. Science 2001;293:1673–1677
13. Kopp E, Ghosh S. Inhibition of NF-kappa B by sodium salicylate and as-
23. Walker BR, Connacher AA, Lindsay RM, Webb DJ, Edwards CR. Carbo-
32. Livingstone DE, Jones GC, Smith K, et al. Understanding the role of glu-
33. Walker BR. Extra-adrenal regeneration of glucocorticoids by 11beta-
34. Walker BR. Extra-adrenal regeneration of glucocorticoids by 11beta-
35. Tomlinson JW, Moore J, Cooper MS, et al. Effects of an 11
40. Best R, Walker BR. Additional value of measurement of urinary cortisol and unconjugated cortisol metabolites in assessing the activity of 11 beta-
41. Rudolph SJ, Petersen KF, Mayerson AB, et al. Mechanism by which high-
44. Tomlinson JW, Sherlock M, Hughes B, et al. Inhibition of 11beta-
48. Wake DJ, Homer NZ, Andrew R, Walker BR. Acute in vivo regulation of 11beta-hydroxysteroid dehydrogenase type 1 activity by insulin and in-